

Attorney Docket No. 1237

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Hartwig Wehrmann	Date:	March 14, 2003
Serial No.:	09/540,578	Group Art Unit:	1638
Filed	March 31, 2000	Examiner:	Ashwin D. Mehta
For:	"INBRED MAIZE LINE PH3AV"		

Assistant Commissioner for Patents  
Washington, D.C. 20231

RULE 132 DECLARATION  
OF  
DR. DINAKAR BHATTRAMAKKI

Sir:

I, Dinakar Bhattramakki, Ph.D., do hereby declare and say as follows:

1. I am skilled in the art of the field of the invention. I have a Ph D. in Plant Molecular Genetics from the University of Illinois at Urbana-Champaign. I have a Bachelor of Science degree in Agricultural Sciences from the University of Agricultural Sciences, Bangalore, India. Since 1997 I have been engaged in the analysis of molecular markers for plants. I have supervised the Molecular Marker Applications lab at Pioneer Hi-Bred International, Inc. from January 2002 until the present.
2. I am familiar with the methods used in the analysis of Simple Sequence Repeat, SSR, marker data for inbred PH3AV conducted at Pioneer Hi-Bred International, Inc. The analysis of the SSR profile of inbred PH3AV may be accomplished without any undue experimentation. The SSR profile for inbred PH3AV is attached hereto.
3. Means of performing this genetic marker profile are well known in the art. SSRs are genetic markers based on polymorphisms in nucleotide sequences. The PCR<sup>TM</sup> detection of SSRs is accomplished by using two oligonucleotide primers flanking the polymorphic segment of DNA. Amplification is accomplished through

Appendix C

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repeated cycles of heat denaturation of the DNA followed by annealing of the primers to their complementary sequences at low temperatures, and extension of the annealed primers with DNA polymerase.

4. Markers are scored following amplification and gel electrophoresis of the amplification products. Scoring of marker genotype is based on the size or weight of the amplified fragment. While variation in the primer used or in laboratory procedures can affect the reported marker score, relative values remain constant regardless of the specific primer or laboratory used.

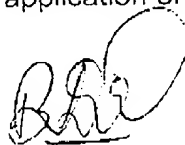
5. Primers that may be used to identify the SSR markers reported herein are publicly available and may be found in the Maize DB on the World Wide Web at [agron.missouri.edu/maps.html](http://agron.missouri.edu/maps.html) (sponsored by the University of Missouri), in Sharopova et al. (Plant Mol. Biol. 48(5-6):463-481) and/or in Lee et al. (Plant Mol. Biol. 48(5-6): 453-461). Markers shown for PH3AV are the publicly available markers in the sources listed above for which PH3AV was tested and shown to be homozygous.

6. Map information is provided by bin number as reported in the Maize DB. The bin number digits to the left of decimal point typically represent the chromosome on which such marker is located, and the digits to the right of the decimal typically represent the location on such chromosome.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date:

By:



Dinakar Bhatramakki

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Public Name of Marker	bin #	PH3AV base pairs
phi427913	1.01	129.38
bnlg1014	1.01	115.95
phi056	1.01	244.05
bnlg1083	1.02	221.86
bnlg1127	1.02	99.21
bnlg1953	1.02	205.80
bnlg1429	1.02	190.00
bnlg1627	1.02	188.57
bnlg1439	1.03	232.00
phi109275	1.03	131.94
phi339017	1.03	145.56
bnlg1203	1.03	306.51
bnlg1484	1.03	143.49
bnlg2086	1.04	224.54
bnlg1886	1.05	141.48
bnlg1057	1.05	247.00
bnlg1041	1.06	240.52
bnlg1615	1.06	223.21
bnlg1556	1.07	198.63
phi323065	1.08	329.53
phi335539	1.08	88.58
phi423298	1.08	133.68
bnlg1331	1.09	117.15
phi011	1.09	226.98
phi308707	1.10	131.11
phi227562	1.11	322.45
phi265454	1.11	217.66
phi064	1.11	80.39
phi402893	2.00	212.91
phi96100	2.01	280.53
bnlg1017	2.02	195.57
bnlg2277	2.02	294.16
bnlg1064	2.03	188.18
bnlg1018	2.04	134.33
bnlg1909	2.05	305.89
bnlg1138	2.06	223.89
bnlg1396	2.06	133.20
bnlg1831	2.06	185.86
phi251315	2.07	123.79
phi328189	2.08	121.35
phi427434	2.08	130.91
phi435417	2.08	214.27
bnlg1141	2.08	152.92
bnlg1940	2.08	247.33
phi127	2.08	122.04
bnlg1520	2.09	312.51

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Public Name of Marker	bin #	PH3AV base pairs
phi101115	3.00	241.89
phi453121	3.00	223.43
phi404206	3.01	300.24
phi193225	3.02	136.71
phi374118	3.02	222.89
bnlg1144	3.02	165.80
bnlg1647	3.02	161.75
bnlg1523	3.03	264.89
bnlg1019	3.03	179.28
bnlg1452	3.04	84.44
bnlg1036	3.05	112.05
phi053	3.05	191.64
phi102228	3.06	135.04
bnlg1160	3.06	222.23
bnlg1951	3.06	126.99
bnlg2241	3.06	142.63
phi072	4.00	139.43
phi213984	4.01	284.60
phi295450	4.01	186.98
bnlg1162	4.03	126.15
phi308090	4.04	218.64
phi096	4.04	234.58
phi438301	4.05	208.45
bnlg1159	4.05	147.81
bnlg1755	4.05	216.63
bnlg1937	4.05	218.18
bnlg1265	4.05	198.45
phi079	4.05	185.40
bnlg1189	4.07	140.17
bnlg2244	4.08	216.18
bnlg1006	5.00	252.16
phi396160	5.02	298.12
phi109188	5.03	161.52
bnlg653	5.04	151.67
phi330507	5.04	131.59
phi331888	5.04	130.72
bnlg1208	5.04	134.77
bnlg1892	5.04	147.78
phi333597	5.05	216.51
phi085	5.06	256.26
bnlg1118	5.07	72.54
bnlg1711	5.07	178.63
phi423796	6.01	128.39
phi389203	6.03	306.15
phi452693	6.04	130.98
phi445613	6.05	100.12
bnlg1174	6.05	218.72

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Public Name of Marker	bin #	PH3AV base pairs
bnlg1759	6.07	136.00
phi070; umc1063	6.07	81.05
phi034	7.02	137.50
bnlg2271	7.03	233.69
phi328175	7.04	121.92
phi250465	7.05	310.75
phi069	7.05	201.72
phi116	7.05	69.69
phi420701	8.00	201.88
bnlg1194	8.02	149.77
phi100175	8.03	144.28
bnlg2082	8.03	172.52
phi115	8.03	302.63
phi121	8.03	97.90
bnlg2046	8.04	326.12
bnlg1176	8.05	251.69
bnlg1152	8.06	148.42
bnlg1056	8.08	108.46
phi015	8.08	81.89
phi233376	8.09	147.93
bnlg2122	9.01	223.46
bnlg1012	9.04	157.54
phi032	9.04	256.63
phi108411	9.05	126.04
phi236654	9.05	122.93
bnlg1375	9.07	185.88
bnlg1129	9.08	298.38
phi041	10.00	202.94
phi96342	10.02	245.07
phi059	10.02	153.35
bnlg1079	10.03	172.22
phi050	10.03	81.29
phi301654	10.04	128.33
phi062	10.04	157.81
phi323152	10.05	141.15
bnlg1074	10.05	161.94
bnlg1185	10.07	151.61
bnlg1450	10.07	181.77
phi109642	2.03/2.04	148.23
bnlg1720	1.09/1.10	236.44